

VISIT...

LANZAROTE
Caliente.COM

CYTOCHROME P4502D6 CATALYZES THE O-DEMETHYLATION OF THE PSYCHOACTIVE ALKALOID IBOGAINE TO 12-HYDROXYIBOGAMINE

R. SCOTT OBACH, JOHN PABLO, AND DEBORAH C. MASH

Department of Drug Metabolism, Central Research Division (R. S. O.), Pfizer, Inc., and Departments of Neurology and Pharmacology (J. P., D. C. M.), University of Miami School of Medicine

(Received January 19, 1998; accepted April 13, 1998)

This paper is available online at <http://www.dmd.org>

ABSTRACT:

Ibogaïne is a psychoactive alkaloid that possesses potential as an agent to treat opiate and cocaine addiction. The primary metabolite arises via O-demethylation at the 12-position to yield 12-hydroxyibogaïne. In this report, evidence is presented that the O-demethylation of ibogaïne observed in human hepatic microsomes is catalyzed primarily by the polymorphically expressed cytochrome P-4502D6 (CYP2D6). An enzyme kinetic examination of ibogaïne O-demethylase activity in pooled human liver microsomes suggested that two (or more) enzymes are involved in this reaction: one with a low K_{Mapp} (1.1 μM) and the other with a high K_{Mapp} (>200 μM). The low K_{Mapp} activity comprised >95% of total intrinsic clearance. Human liver microsomes from three individual donors demonstrated similar enzyme kinetic parameters (mean K_{Mapp} = 0.55 $\pm$ 0.09 μM and 310 $\pm$ 10 μM for low and high K_{M} activities, respectively). However, a fourth human microsome sam-

ple that appeared to be a phenotypic CYP2D6 poor metabolizer possessed only the high K_{Mapp} activity. In hepatic microsomes from a panel of human donors, the low K_{Mapp} ibogaïne O-demethylase activity correlated with CYP2D6-catalyzed bufuralol 1'-hydroxylase activity but not with other P450 isoform-specific activities. Quinidine, a CYP2D6-specific inhibitor, inhibited ibogaïne O-demethylase (IC_{50} = 0.2 μM), whereas other P450 isoform-specific inhibitors did not inhibit this activity. Also, of a battery of recombinant heterologously expressed human P450 isoforms, only rCYP2D6 possessed significant ibogaïne O-demethylase activity. Thus, it is concluded that ibogaïne O-demethylase is catalyzed by CYP2D6 and that this isoform is the predominant enzyme of ibogaïne O-demethylation in humans. The potential pharmacological implications of these findings are discussed.

Ibogaïne (fig. 1) is a psychoactive alkaloid isolated from the root of *Tabernanthe iboga*, a shrub native to Africa. The plant has been used by native peoples for such purposes ranging from the alleviation of fatigue, thirst, and hunger to its use in greater quantities as a hallucinogen in religious rituals. More recently, ibogaïne has been explored as an agent that combats the symptoms of drug withdrawal (Goutarel *et al.*, 1993; Lotsof, 1985; Mash *et al.*, 1996). Additionally, preclinical studies of ibogaïne in rodent models of cocaine and opiate self-administration support the notion that it is an anti-addictive agent (Aceto and Harris, 1991; Cappendijk and Dzoljic, 1993; Glick *et al.*, 1992a, 1992b; Serhsen *et al.*, 1994, 1997).

The cytochromes P450 (P450)¹ constitute a large family of enzymes present throughout the animal and plant kingdoms (Nelson, 1995). The various enzymes function in the metabolism of endogenous and exogenous compounds. For the latter, it is maintained that P450-catalyzed oxidation reactions serve to impart greater polarity to xenobiotics, thereby making them more readily excreted. Also, P450-catalyzed reactions usually result in metabolites that are more amenable to conjugation reactions (*e.g.* glucuronidation, sulfation, etc.) that result in more polar and more readily excreted compounds. In humans, over 20 isoforms of P450 have been characterized, many of which have been shown to be involved in the oxidative metabolism of

drugs and other xenobiotics. A human isoform of interest is CYP2D6. This isoform is involved in the metabolism of numerous neuroleptic agents, β -blockers, tricyclic antidepressants, and opioids (Eichelbaum and Gross, 1990; Fromm *et al.*, 1997). Several investigators have developed a pharmacophore for CYP2D6 substrates, common elements of these being that substrates possess an amino nitrogen (or other cationic center) and a site for P450-catalyzed oxidation 5 to 7 Å away (deGroot *et al.*, 1997; Islam *et al.*, 1991; Koymans *et al.*, 1992; Strobl *et al.*, 1993). An important aspect of the CYP2D6 isoform is that it is subject to polymorphic expression, particularly in Caucasians (Gonzalez and Meyer, 1991). Approximately 5–10% of Caucasians lack a functional copy of the CYP2D6 gene and hence lack the enzyme. Such individuals are termed poor metabolizers, owing to their decreased capacity to metabolize and clear CYP2D6 substrates. Such individuals are often subject to a higher incidence of adverse drug reactions due to elevated drug concentrations. Also, for drugs that require CYP2D6-catalyzed bioactivation to a pharmacologically active metabolite (*e.g.* codeine $\rightarrow$ morphine), efficacy can be reduced in poor metabolizer subjects.

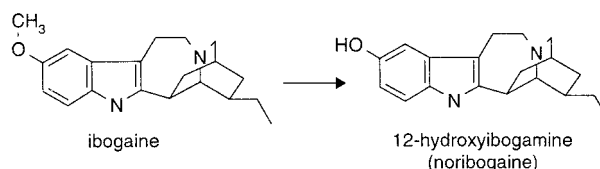


FIG. 1. Structures of ibogaïne and 12-hydroxyibogaïne.

¹ Abbreviation used is: P450, cytochrome P450.

As ibogaine represents a potentially useful therapeutic agent in the treatment of opiate and psychostimulant addiction and opiate withdrawal, knowledge concerning the enzymes involved in the metabolism of this compound in humans is important. Thus, these experiments were undertaken to identify P450 isoforms involved in the metabolism of ibogaine to its *O*-demethylated metabolite, 12-hydroxyibogamine (fig. 1).

Materials and Methods

Reagents and Biological Materials. Ibogaine and 12-hydroxyibogamine (noribogaine) were obtained from s.a. Omnicem Corp. (Belgium), and the deuterated internal standard of 12-hydroxyibogamine was obtained from the Medications Development Division of NIDA. Commercial sources were as follows: quinidine (Aldrich), sulfaphenazole and furaphylline (Ultrafine Pure Chemicals, Ltd.), and ketoconazole (Janssen Biotech NV). Human liver microsomes were prepared from human liver samples using standard procedures and were characterized for P450 isoform-specific activities using standard methods of catalytic activity measurement: tolbutamide hydroxylase for CYP2C9 (Miners *et al.*, 1988), *S*-mephenytoin hydroxylase for CYP2C19 (Meier *et al.*, 1985), bufuralol 1'-hydroxylase for CYP2D6 (Kronbach *et al.*, 1987), testosterone 6 β -hydroxylase for CYP3A (Sonderfan *et al.*, 1987), and phenacetin *O*-deethylase for CYP1A2 (Butler *et al.*, 1989). Heterologously expressed P450 isoforms were obtained from either Gentest Corp. (Woburn, MA) or the Molecular Genetics Department, Pfizer Central Research (Groton, CT). Assay for protein was accomplished using the BCA assay kit (Pierce) using bovine serum albumin as a standard, and assay for P450 was conducted using a standard method (Omura and Sato, 1964). Specific content of human liver microsomal preparations were 0.31, 0.11, 0.20, and 0.23 nmol P450/mg microsomal protein for HL-1000-3, HL-1021, HL-1028, and HL-1032, respectively. The pooled human liver microsome preparation consisted of equal mixtures of preparations from ten individual donors (including none of the four individual samples listed above). The P450 content was 0.21 nmol/mg microsomal protein.

Assay of Ibogaine *O*-Demethylase Activity. Incubation mixtures contained liver microsomes (0.5 mg protein/ml), ibogaine (0.1–500 μ M), MgCl₂ (3.3 mM), and NADPH (1.3 mM) in a total volume of 1.0 ml of potassium phosphate buffer (25 mM, pH 7.5). Incubations were commenced with the addition of NADPH and shaken open to air in a water bath set at 37°C. Initial time course experiments demonstrated reaction velocity linearity out to 20 min and reaction velocity linearity with protein concentrations of up to 2.0 mg/ml. Thus, all subsequent experiments were conducted with an incubation time of 20 min or less and protein concentrations below 2.0 mg/ml. Incubations were terminated with the addition of 2 ml of ice-cold Na₂CO₃, pH 10, and were frozen prior to analysis. 12-Hydroxyibogamine concentrations were determined using a method involving extraction, chemical derivatization, and GC-MS as previously described (Hearn *et al.*, 1995) or using an HPLC-MS method as described below when additional assay sensitivity was needed. Incubations using recombinant heterologously expressed P450 isoforms (rCYP) were conducted in a similar manner except that microsomal protein concentrations were adjusted for each expressed isoform to account for differences in expression level. Protein concentrations ranged between 0.07 mg/ml (for CYP2D6) to 0.62 mg/ml (for CYP2C19). For the rCYP2D6 substrate saturation experiment, the incubation volume was increased to 5.0 ml to permit quantitation of product in incubations containing low ibogaine concentrations. Inhibition experiments were conducted using pooled human liver microsomes in the presence of quinidine (0.1–3.0 μ M), ketoconazole (0.1–3.0 μ M), sulfaphenazole (0.1–3.0 μ M), or furaphylline (1.0–100 μ M).

HPLC-MS Assay of 12-Hydroxyibogamine. Samples were extracted as previously described (Hearn *et al.*, 1995), and the evaporated ethyl acetate extract was reconstituted in 75 μ l of HPLC mobile phase. The mobile phase composition was 20 mM CH₃COOH (adjusted to pH 4.0 with NH₄OH) in 32% CH₃CN. Samples (50 μ l) were injected onto a Waters Symmetry C18 5 μ m (3.9 $\times$ 150 mm) column at a flow rate of 0.8 ml/min. The effluent was introduced into an APCI source of a Sciex API100 mass spectrometer operated in the positive ion mode. The source temperature was 500°C, and the orifice voltage was 35 V. Other settings and state file parameters were adjusted to optimize the signal. Detection was accomplished by selected ion monitoring of

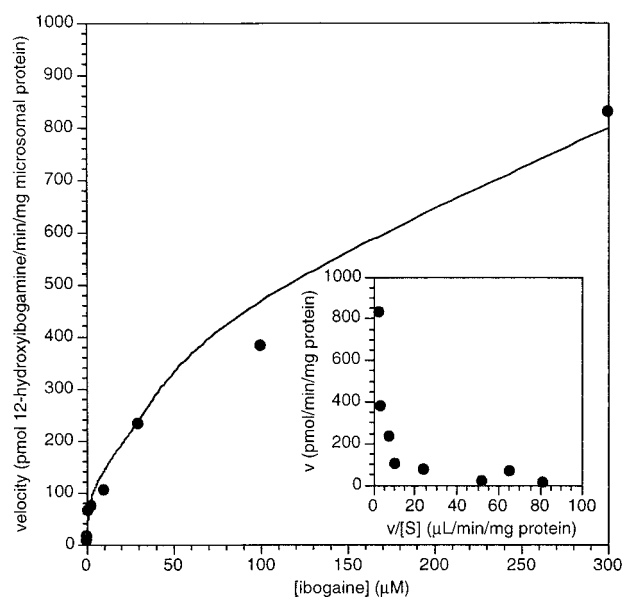


Fig. 2. Substrate saturation plot for ibogaine *O*-demethylase in pooled human liver microsomes.

Ibogaine (0.1–300 μ M) was incubated with human liver microsomes pooled from ten individual donors (0.5 mg/ml), and 12-hydroxyibogamine was determined as described in *Materials and Methods*. Each point represents the mean of triplicate determinations. *Inset*, Eadie-Hofstee plot.

m/z 297 (12-hydroxyibogamine) and *m/z* 299 ([²H₂]12-hydroxyibogamine internal standard). The analytes eluted at 1.8 min. The dynamic range of this assay was from 3.0 to 1000 ng/ml using a 1-ml sample aliquot.

Results and Discussion

Human liver microsomes catalyze the *O*-demethylation of ibogaine. In initial time course experiments, both the consumption of ibogaine and the formation of 12-hydroxyibogamine were measured. The *O*-demethylation reaction was the major route of metabolism. Of the ibogaine consumed, 75–80% was accounted for as 12-hydroxyibogamine (data not shown).

Substrate saturation experiments for ibogaine *O*-demethylase activity conducted in pooled human liver microsomes suggested the presence of two kinetically distinguishable activities as observed on an Eadie-Hofstee plot (fig. 2). The low K_{Mapp} activity contributed the majority of intrinsic clearance with kinetic parameters of 1.1 μ M and 106 pmol/min/mg microsomal protein for K_{Mapp} and V_{max} , respectively (table 1). For the high K_{Mapp} activity, values of 250 μ M and 1260 pmol/min/mg microsomal protein were obtained for K_{Mapp} and V_{max} , respectively. Scaling the sum of the *in vitro* Cl'_{int} values to reflect an *in vivo* Cl'_{int} (Obach *et al.*, 1997) suggests that ibogaine is a high intrinsic clearance compound (*in vivo* Cl'_{int} = 90 ml/min/kg) in humans.

Human liver microsomes from four individual donors were also examined to assess interindividual variability; these included one sample from a putative CYP2D6 poor metabolizer (HL-1032). For three of the four, biphasic enzyme kinetics were observed (as assessed through Eadie-Hofstee plots of the data) with low and high mean K_{Mapp} values of 0.55 and 310 μ M (table 1). The fourth donor sample (HL-1032) lacked the low mean K_{Mapp} activity.

Upon observation that the low K_{Mapp} activity contributed the major portion of ibogaine intrinsic clearance through the *O*-demethylase pathway, subsequent experiments designed to identify the P450 isoform responsible for this activity were conducted at low substrate concentrations (1.0 μ M). Measurement of ibogaine *O*-demethylase in

TABLE 1

Summary of enzyme kinetic parameters of ibogaine O-demethylase activity in pooled and individual human liver microsomes and recombinant heterologously expressed CYP2D6^a

Sample	Human Pool	HL-1021	HL-1028	HL-1000-3	HL-1032	Mean $\pm$ SD ^b	rCYP2D6
K_{Mapp} (1)	1.1	0.46	0.57	0.63	—	0.55 ± 0.09	0.19
V_{max} (1)	106	53	127	106	—	95 ± 38	12.1^d
Cl'_{int} (1)	0.096	0.12	0.22	0.17	—	0.17 ± 0.05	0.064^d
K_{Mapp} (2)	250	320	290	320	310	310 ± 10	—
V_{max} (2)	1260	1500	1470	1650	1480	1520 ± 80	—
Cl'_{int} (2)	0.005	0.005	0.005	0.005	0.005	0.005 ± 0.000	—
sum Cl'_{int}	0.10	0.13	0.23	0.18	0.005	0.18 ± 0.05^c	—

^a Units are as follows: K_{Mapp} , μM ; V_{max} , pmol/min/mg microsomal protein; Cl'_{int} , ml/min/mg microsomal protein.

^b Mean $\pm$ SD of the human liver microsome samples from four individual donors.

^c Mean $\pm$ SD not including HL-1032 (poor metabolizer).

^d For rCYP2D6, units are as follows: V_{max} , pmol/min/pmol CYP2D6; Cl'_{int} , ml/min/pmol CYP2D6.

liver microsomes from a panel of 19 individual donors resulted in activities ranging up to 85 pmol/min/mg protein. Attempts at correlation of these activities with standard P450 isoform-specific activities (CYP1A2 phenacetin O-deethylase, CYP2C9 tolbutamide hydroxylase, CYP2C19 mephenytoin hydroxylase, CYP2D6 bufuralol 1'-hydroxylase, and CYP3A testosterone 6 β -hydroxylase) demonstrated a correlation only with CYP2D6-catalyzed bufuralol 1'-hydroxylase ($r^2 = 0.711$; fig. 3, table 2).

P450 isoform-specific inhibitors furaphylline (CYP1A2), ketoconazole (CYP3A), sulfaphenazole (CYP2C9), and quinidine (CYP2D6) were examined for their ability to inhibit ibogaine O-demethylation at a substrate concentration of 1.0 μM . Of the four inhibitors, only quinidine inhibited this reaction, with an IC_{50} value of 0.2 μM . This inhibitory potency of quinidine is consistent with inhibition of CYP2D6 (Strobl *et al.*, 1993). Ibogaine O-demethylase activities at quinidine concentrations above 1 μM were under the limit of detection, indicating that CYP2D6 is exclusively involved in this activity in human liver microsomes at a low ibogaine concentration.

To confirm the involvement of CYP2D6 in the metabolism of ibogaine to 12-hydroxyibogamine, several heterologously expressed rCYP isoforms were examined for this activity. Of the isoforms examined, rCYP2D6, rCYP3A4, and rCYP2C19 demonstrated measurable ibogaine O-demethylase activity (table 3). Substrate saturation experiments were conducted with rCYP2D6 to determine whether the K_{Mapp} was close to the low K_{Mapp} value measured in human liver microsomes (fig. 4, table 1). The K_{Mapp} value of 0.19 μM was somewhat lower than that measured in human liver microsomes. However, it is not uncommon to measure slightly disparate K_{Mapp} values in heterologously expressed rCYP isoforms and liver microsomes. It is interesting to note that the K_{Mapp} values measured for ibogaine O-demethylase in this work are close to the reported K_i value for ibogaine on CYP2D6-mediated bufuralol 1'-hydroxylase in human liver microsomes (0.4 μM ; Fonne-Pfister and Meyer, 1988). The V_{max} value of 12.1 pmol/min/pmol P450 is substantially higher than corresponding low V_{max} values in human liver microsomes after normalization to P450 content (range of 0.34 to 0.64 pmol/min/pmol P450). This is consistent with the notion that CYP2D6 comprises a small portion (<5%) of total liver microsomal P450 content.

The evidence presented strongly supports the notion that the O-demethylation of ibogaine is primarily catalyzed by CYP2D6 in human liver microsomes. All three approaches (correlation analysis, P450-specific inhibitors, heterologously expressed rCYP isoforms) provided results that were in agreement. The identity of the high K_{Mapp} P450 isoform was not determined but could be CYP3A4 or CYP2C19, as these two heterologously expressed enzymes were observed to catalyze the reaction to a small extent. The importance of CYP2D6 has several important potential implications for the clinical

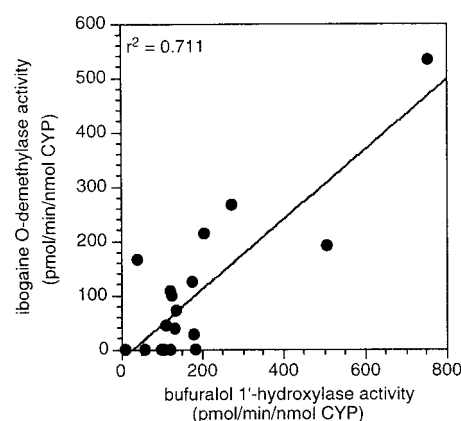


FIG. 3. Correlation between bufuralol 1'-hydroxylase activity and ibogaine O-demethylase activity in a panel of human liver microsomes.

Ibogaine (1.0 μM) was incubated with human liver microsomes from 19 individual donors (0.5 mg/ml), and 12-hydroxyibogamine was determined as described in Materials and Methods. Each point represents the average of duplicate determinations.

TABLE 2

Correlation between ibogaine O-demethylase activity and standard P450 isoform-specific activities in a panel of human liver microsome samples^a

P450 Isoform	Activity	Correlation Coefficient with Ibogaine O-Demethylase (r^2)
CYP1A2	Phenacetin O-deethylase	0.009
CYP2C9	Tolbutamide hydroxylase	0.295
CYP2C19	Mephenytoin hydroxylase	0.002
CYP2D6	Bufuralol 1'-hydroxylase	0.711 ^b
CYP3A	Testosterone 6 β -hydroxylase	0.070

^a Ibogaine (1.0 μM) was incubated with human liver microsomes from 19 individual donors (0.5 mg/ml), each in duplicate. Activities were normalized to P450 content and correlated with isoform-specific marker activities measured using standard methods.

^b $p < 0.0001$.

pharmacology of this agent. First, because this major route of ibogaine metabolic clearance is mediated by the CYP2D6 isoform, pharmacogenetic differences in the response to this compound are expected to be observed. CYP2D6 poor metabolizers would be expected to be subject to greater ibogaine exposures, especially after oral administration, than extensive metabolizers. Preliminary experiments in humans suggest that systemic exposure to ibogaine and 12-hydroxyibogamine are substantially different between CYP2D6 extensive and poor metabolizer subjects (D. Mash, unpublished observations). Whether this difference will be great enough to elicit adverse drug reactions in poor metabolizers administered doses deemed efficacious in extensive metabolizers remains to be determined. This is further

TABLE 3

Ibogaïne O-demethylase activities of recombinant heterologously expressed P450 isoforms^a

P450 Isoform	Ibogaïne O-Demethylase nmol/min/nmol P450
CYP1A1	ND ^b
CYP1A2	ND ^b
CYP2A6	ND ^b
CYP2C9	ND ^b
CYP2C19	0.09
CYP2E1	ND ^b
CYP2D6	7.97
CYP3A4	0.12
CYP3A5	ND ^b

^a Ibogaïne (1.0 μ M) was incubated with microsomes containing heterologously expressed recombinant CYP enzymes. Each value represents the average of two determinations.

^b ND, no detectable activity.

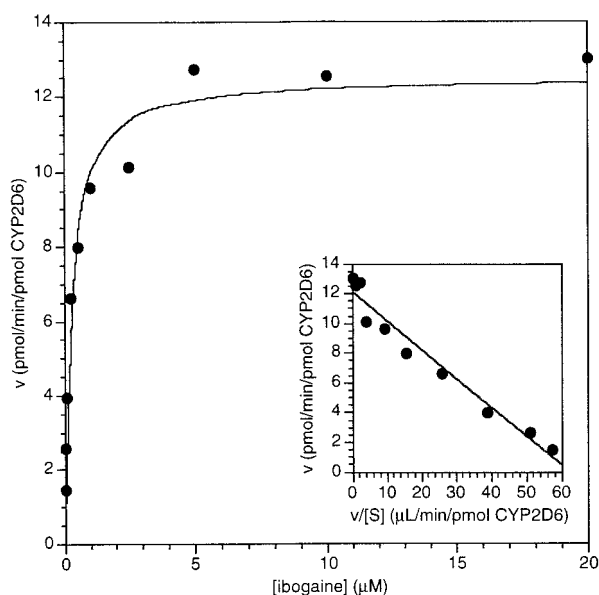


Fig. 4. Substrate saturation plot for ibogaïne O-demethylase by heterologously expressed recombinant human CYP2D6.

Ibogaïne (0.025–20 μ M) was incubated with microsomes containing heterologously co-expressed CYP2D6 and NADPH/P450 oxidoreductase (0.14 mg/ml), and 12-hydroxyibogaïne was determined as described in *Materials and Methods*. The incubation volume was 5.0 ml to permit measurement of product at the low substrate concentrations. Each point represents the mean of triplicate determinations. Inset, Eadie-Hofstee plot.

complicated by the observation that ibogaïne and 12-hydroxyibogaïne demonstrate some differences in pharmacological profile (Staley *et al.*, 1996). Furthermore, owing to the fact that CYP2D6-catalyzed ibogaïne O-demethylase activity is characterized by a low K_{Mapp} value, the compound could exhibit oral dose supraproportional exposure due to saturation of first-pass metabolism. Such a phenomenon has been observed with other CYP2D6 substrates such as propafenone (Siddoway *et al.*, 1987) and paroxetine (Sindrup *et al.*, 1992). However, other factors such as the dose, extent of plasma binding, and absorption rate constant also contribute to this phenomenon, so that it cannot be predicted from K_{Mapp} values alone.

Many CYP2D6 substrates are subject to drug interactions. For example, quinidine, a potent CYP2D6 inhibitor, can inhibit the metabolism of the CYP2D6 substrate desipramine *in vivo* to the extent that extensive metabolizer subjects receiving quinidine demonstrate desipramine pharmacokinetics phenotypically similar to those exhibited in CYP2D6 poor metabolizers (Broten *et al.*, 1987). The common

antidepressant fluoxetine, also a potent inhibitor of CYP2D6 activity, alters the metabolism of dextromethorphan in human subjects (Otton *et al.*, 1993). In consideration that the potential patient population that would benefit from the therapeutic effects of ibogaïne are likely to have taken other medications (prescription and/or illicit) that are CYP2D6 substrates and inhibitors, the potential for drug interactions with ibogaïne is increased.

The product of ibogaïne O-demethylation, 12-hydroxyibogaïne, has been demonstrated to possess pharmacologic activity. *In vitro* radioligand binding assays conducted to identify the potency and selectivity profiles for ibogaïne and 12-hydroxyibogaïne have demonstrated that the metabolite has a binding profile that is similar, but not identical to, the parent drug (Pablo and Mash, 1998; Staley *et al.*, 1996). 12-Hydroxyibogaïne demonstrated the highest potency values at the cocaine recognition site on the serotonin transporter (Mash *et al.*, 1995a; Staley *et al.*, 1996). Ibogaïne and 12-hydroxyibogaïne were equipotent at vesicular monoamine and dopamine transporters, whereas the metabolite demonstrated higher affinity at the kappa-1 and mu opioid receptors and lower affinity at the NMDA receptor complex (Mash *et al.*, 1995b; Pablo and Mash, 1998; Staley *et al.*, 1996). The desmethyl metabolite has been shown recently to be a full agonist at the mu opioid receptor (Pablo and Mash, 1998). The *in vivo* activity of the metabolite as a full mu agonist may explain the ability of ibogaïne to block the acute signs of opiate withdrawal in humans and its suppressive effects on morphine self-administration in rodents. Because the precise mix of molecular targets important for the anti-addictive effects are not definitively known, the relative contributions of ibogaïne and 12-hydroxyibogaïne to the actions of ibogaïne *in vivo* has yet to be well established at the biochemical level. However, because CYP2D6 has been demonstrated to be present in the brain (Tyndale *et al.*, 1991), it is compelling to hypothesize that some or all of the CNS activity of ibogaïne may be the result of 12-hydroxyibogaïne generated *in situ* in the brain. (Such a hypothesis also exists for the CYP2D6-catalyzed metabolism of codeine to the active metabolite morphine [Sindrup *et al.*, 1996]). This effect would have important implications for the pharmacological activity of ibogaïne in CYP2D6 extensive and poor metabolizers. Regardless of this hypothesis, the evidence presented in this report suggest that the CYP2D6 phenotype may prove to be an important determinant in the clinical pharmacology of ibogaïne and that it may be necessary to determine CYP2D6 metabolizer status in subjects administered this compound.

Acknowledgments. The authors extend their gratitude to Dr. Donald Tweedie and associates (Drug Metabolism Department, Pfizer) for generation and characterization of the human liver microsomes used in these experiments and to Dr. Stafford McLean (Neuroscience Department, Pfizer) for initial discussions of this work. D. C. M. and J. P. are supported by the Addiction Research Fund.

References

- Aceto MD and Harris LS (1991) Dependence studies of compounds in the rhesus monkey and the mouse. *Natl Inst Drug Abuse Res Monogr Ser* **119**:522–523.
- Broten K, Gram LF, Haghfelt T and Bertilsson L (1987) Extensive metabolizers of debrisoquine become poor metabolizers during quinidine treatment. *Pharmacol Toxicol* **60**:312–314.
- Butler MA, Guengerich FP and Kadlubar FF (1989) Metabolic oxidation of the carcinogen 4-aminobiphenyl and 4,4'-methylene bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Res* **49**:25–31.
- Cappendijk SLT and Dzoljic MR (1993) Inhibitory effects of ibogaïne on cocaine self-administration in rats. *Eur J Pharmacol* **241**:261–265.
- deGroot MJ, Bijloo GJ, Martens BJ, vanAcker FAA and Vermeulen NPE (1997) A refined substrate model for human cytochrome P450 2D6. *Chem Res Toxicol* **10**:41–48.
- Eichelbaum M and Gross AS (1990) The genetic polymorphism of debrisoquine/sparteine metabolism: Clinical aspects. *Pharmacol Ther* **46**:377–394.
- Fonne-Pfister R and Meyer U (1988) Xenobiotic and endobiotic inhibitors of the cytochrome P-450db1 function, the target of the debrisoquine/sparteine type polymorphism. *Biochem Pharmacol* **37**:3829–3835.
- Fromm MF, Kroemer HK and Eichelbaum M (1997) Impact of P450 genetic polymorphism on

- the first-pass extraction of cardiovascular and neuroactive drugs. *Adv Drug Del Rev* **27**:171–199.
- Glick SD, Rossman K, Rao NC, Maisonneuve IM and Carlson JN (1992a) Effects of ibogaine on acute signs of morphine withdrawal in rats: Independence from tremor. *Neuropharmacology* **31**:497–500.
- Glick SD, Rossman K, Steindorf S, Maisonneuve IM and Carlson JN (1992b) Effects of and aftereffects of ibogaine on morphine self-administration in rats. *Eur J Pharmacol* **195**:341–345.
- Gonzalez FJ and Meyer UA (1991) Molecular genetics of the debrisoquine-sparteine polymorphism. *Pharmacol Ther* **50**:233–238.
- Goutarel R, Gollnhofer O and Sillans R (1993) Pharmacodynamics and therapeutic applications of iboga and ibogaine in psychotherapy and for controlling narcotic dependence. *Psychodelic Monogr Essays* **6**:71–111.
- Hearn WH, Pablo J, Hime GW and Mash DC (1995) Identification and quantitation of ibogaine and an *O*-demethylated metabolite in brain and biological fluids using gas chromatography-mass spectrometry. *J Anal Toxicol* **19**:427–434.
- Islam SA, Wolf CR, Lennard MS and Sternberg MJE (1991) A three-dimensional molecular template for substrates of human cytochrome P450 involved in debrisoquine 4-hydroxylation. *Carcinogenesis* **12**:2211–2219.
- Koymans L, Vermeulen NPE, van Acker SABE, teKoppele JM, Heykants JJP, Lavrijsen K, Meuldermans W and Donne-Op den Kelder GM (1992) A predictive model for substrates of cytochrome P450-debrisoquine (2D6). *Chem Res Toxicol* **5**:211–219.
- Kronbach T, Mathys D, Gut J, Catin T and Meyer UA (1987) High-performance liquid chromatographic assays for bufuralol 1'-hydroxylase, debrisoquine 4-hydroxylase, and dextromethorphan *O*-demethylase in microsomes and purified cytochrome P-450 isozymes of human liver. *Anal Biochem* **162**:24–32.
- Lotsof HS (1985) Rapid method for interrupting the narcotic addiction syndrome. U.S. Patent 4,499,096.
- Mash DC, Staley JK, Baumann MH, Rothman RB and Hearn WL (1995a) Identification of a major metabolite of ibogaine that targets serotonin transporters and elevates serotonin. *Life Sci* **57**:PL45–PL50.
- Mash DC, Pablo J, Staley JK, Holohean AM, Hackman JC and Davidoff RA (1995b) Properties of ibogaine and a principal metabolite (12-hydroxyibogamine) at the MK-801 binding site on the NMDA receptor complex. *Neurosci Lett* **192**:53–56.
- Mash DC, Sanchez-Ramos J and Hearn WL (1996) Noribogaine compounds for treating chemical dependency in mammals. U.S. Patent WO 9603127.
- Meier UT, Kronbach T and Meyer UA (1985) Assay of mephenytoin metabolism in human liver microsomes by high performance liquid chromatography. *Anal Biochem* **151**:286–291.
- Miners JO, Smith KJ, Robson RA, McManus ME, Veronese ME and Birkett DJ (1988) Tolbutamide hydroxylation by human liver microsomes: Kinetic characterization and relationship to other cytochrome P-450 dependent xenobiotic oxidations. *Biochem Pharmacol* **37**:1137–1144.
- Nelson DR (1995) Cytochrome P450 nomenclature and alignment of selected sequences, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ortiz de Montellano PR ed) pp 575–606, Plenum Press, New York.
- Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ and Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. *J Pharmacol Exp Ther* **283**:46–58.
- Omura T and Sato R (1964) The carbon monoxide binding pigment of liver microsomes. *J Biol Chem* **239**:3137–3142.
- Otton SV, Wu D, Joffe RT, Cheung SW and Sellers EM (1993) Inhibition by fluoxetine of cytochrome P4502D6 activity. *Clin Pharmacol Ther* **53**:401–407.
- Pablo J and Mash DC (1998) Noribogaine stimulates naloxone-sensitive [³⁵S]GTPγS binding. *NeuroReport* **9**:109–114.
- Sershen H, Hashim A and Lojtha A (1994) Ibogaine reduces preference for cocaine consumption in C57BL/6By mice. *Pharmacol Behav* **47**:13–19.
- Sershen H, Hashim A and Lojtha A (1997) Ibogaine and cocaine abuse: Pharmacological interactions at dopamine and serotonin receptors. *Brain Res Bull* **42**:161–168.
- Siddoway LA, Thompson KA, McAllister CB, Wang T, Wilkinson GR, Roden DM and Woosley RL (1987) Polymorphism of propafenone metabolism and disposition in man: Clinical pharmacokinetic consequences. *Circulation* **75**:785–791.
- Sindrup SH, Brosen K and Gram LF (1992) Pharmacokinetics of the selective serotonin reuptake inhibitor paroxetine: Nonlinearity and relation to the sparteine oxidation polymorphism. *Clin Pharmacol Ther* **51**:288–295.
- Sindrup SH, Hoffman U, Asmussen J, Mikus G, Brosen K, Nielsen F, Ingwersen SH and Broen Christensen C (1996) Impact of quinidine on plasma and cerebrospinal fluid concentrations of codeine and morphine after codeine intake. *Eur J Clin Pharmacol* **49**:503–509.
- Sonderfan AJ, Arlotto MP, Dutton DR, McMillen SK and Parkinson A (1987) Regulation of testosterone hydroxylation by rat liver microsomal P-450. *Arch Biochem Biophys* **255**:27–41.
- Staley JK, Ouyang Q, Pablo J, Hearn WL, Flynn DD, Rothman RB, Rice KC and Mash DC (1996) Pharmacological screen for activities of 12-hydroxyibogamine: A primary metabolite of the indole alkaloid ibogaine. *Psychopharmacology* **127**:10–18.
- Strobl GR, von Kruegener S, Stockigt J, Guengerich FP and Wolff T (1993) Development of a pharmacophore for inhibition of human liver cytochrome P-450 2D6: Molecular modeling and inhibition studies. *J Med Chem* **36**:1136–1145.
- Tyndale RF, Sunahara R, Inaba T, Kalow W, Gonzalez FJ and Niznik HB (1991) Neuronal cytochrome P450IID1 (debrisoquine/sparteine-type): Potent inhibition of activity by (–)-cocaine and nucleotide sequence identity to human hepatic P450 gene CYP2D6. *Mol Pharmacol* **40**:63–68.